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THE ORIGIN AND DEVELOPMENT OF THE EMBRYO-SAC OF SALVIA SPLENDENS

by

ANNA LIVINGSTON BURTON

A Thesis Submitted for the Degree of

MASTER OF ARTS

UNIVERSITY OF WISCONSIN

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HISTORICAL SUMMARY.

The first mention of the membrane surrounding the embryo-sac of angiosperms was by Malpighi (17), who, in 1671, described it and called it the vesicula colliquamenti or amnion. Considering the early date, the drawings accompanying show a high degree of accuracy.

Nehemiah Grew (8) seems to refer to the same membrane as the Inmost, Innermost or Inner Membrane, also as the Inmost Cover.

From the time of Malpighi until long after 1800, it was thought that the embryo originated from entire pollen grains which had been transported, after falling on the stigma, through the canal of the pistil, to the sac itself. So much stress came to be placed upon this explanation of the origin of the embryo that the embryo-sac received but little attention.

While struggling with this question and this method of answering it, however, botanists were finally led to make closer study of the seed structure and the development of the embryo itself.

Du Trochet (6) did much work along both of these lines and aided in laying a firm foundation for plant embryology. He called the membrane surrounding the embryo the Perisperme immédiat (unmittelbares Perisperm) or Tegmen.

Brown (4) referred to a membrane, the amande, as ordinarily forming the interior membrane of the grain. But in a small number of plants there is inside of this, a tunique additionnelle, which is the vesicula colliquamenti or amnion of Malpighi. Usually this amnion distends until it pushes aside the amande or absorbs all the substance of it, by the time that the embryo and albumen of the seed are present. But he thought that when albumen is present, this membrane proper is gradually obliterated and replaced by cells of the amande. In some cases the albumen is a store of granular substance in the cells of the amande, in which case the amnion persists, forming a "tunic proper" for the embryo.

In speaking of the Nymphaeaceae, he wrote that the sac which envelops the embryo contains a pulpy semi-fluid which he named vitellus, a name he applied to all substances interposed between the albumen and the embryo.

Brongniart (3), in his study of the seed parts, found the amande a uniformly spongy tissue composed of two distinct parts. The external part is cellular and white and contains a great number of very small, white globules. [This external part, he says, is the chorion of Malpighi.] In its interior floats a sac membraneux [probably the embryo-sac] at first quite free, but later, after extend-

ing to the chalaza, seems to become attached to it. The wall of this sac, when it has reached a certain stage of development, consists of a single layer of very small cells very different from the surrounding cells. The cavity of the sac is filled with a liquid in which small globules float. This, Brongniart believed, is the liquid that Malpighi called the liquor of the amnion.

Mirbel (19) gave the name fifth envelope, or quintine to this membrane, for he considered the envelopes of the quite mature ovule in their order, beginning with the outer, as outer and inner integuments, nucellus and perisperm and lastly the fifth envelope just mentioned.

Crude as the work preceding had been, it formed a basis for the real study of embryo-sac structure, begun by Meyen (18). He says: "In Figs. 35 and 36, Pl.XIII are the Keimbläschen, one day after the resulting formation produced from the sperm of Orchis Morio, and one sees that the lower end of the Keimbläschen, changed to an articulate thread, swells spherically; in these single spherical cells are formed two new cells with their nuclei, and these cells are those which form the embryo. The Keimbläschen after this do not originate the embryo, but from the same, in consequence of peculiar nourishment inside the Keimsack [embryo-sac] goes forth first a formation, which in most

cases is produced as a single spherical cell, and out of which the embryo is formed." He farther describes a *Bläschen* [vesicle] as being small, pear-shaped, transparent, and granular and lying at the micropylar end of the embryo-sac. This appears to describe the egg, especially when he says that later it has been noticed [by Brongniart] to become cellular, due to fertilization.

But Meyen thinks the *Bläschen* itself a *product* of fertilization, a result of the union of the pollen tube point with the slimy mass at the micropylar end of the sac; the *Bläschen*, he believes, through the formation of cells within, gradually changes to the thread-like form mentioned above.

His drawings to represent these steps are not very easily followed, partly, probably, because his writings and drawings show the influence of his incorrect notion of fertilization. His Fig. 6, Pl. XIV shows well the embryo-sac itself, the *Eyweisskörper* [endosperm] and embryo, with its *suspensor*.

Hofmeister (14) gives excellent drawings of the micropylar end of the sac, sometimes including what appears to be the *primary endosperm nucleus*. He says that he finds in the micropylar end two to four free-swimming nuclei, each sometimes surrounded by a cell wall, sometimes not.

One of these [probably the egg nucleus] forms itself into a cell and becomes pear-shaped, with its rounding end touching the membrane of the sac. Its other end hangs free. This, he says, is the Keimbläschen of Meyen, and is the place of origin of the embryo. Hofmeister calls it the Ey [egg] of the plant. He sometimes finds three Keimbläschen present.

Immediately there appears in the sac [the embryo-sac of Godetia quadrivulnera] near its narrowing end, a nucleus with a distinct membrane and a large nuclear body. From his figures, probably this is the fusion nucleus derived from the two polar nuclei. He thinks that, after the passage of the pollen tube substance, by osmotic streaming, through the tube wall and sac wall and, after its arrival at the Keimbläschen wall, it enters this egg cell by endosmosis,to produce fertilization. A nucleus originates from the heaping up of the protoplasm and from this the embryo originates.

The same author (15) describes the origin of the ovule and the embryo-sac. In his Pl. II, Fig.2, he lacks but one polar nucleus of showing a complete eight-nucleate embryo-sac; he shows antipodal cells at different times, but says very little about them, excepting to refer to them as the cells in the chalazal end of the sac. In

Pl. VI, Fig. 3, there is strong evidence that he may have seen fusing polar nuclei.

The Eykern [megaspore mother cell] is shown in his Pl. VIII, Fig. 1; this same plate has other drawings which show that the nucleus or nuclei of the sac had divided. In this volume are many carefully traced cell-divisions of the embryo. The Eyweisskörper [endosperm] is referred to several times.

Hofmeister (16), in another work (although discussing free cell formation), gives good descriptions of the parts of the embryo-sac. What he here calls the Keimbläschen are the cells of the egg apparatus. The synergids are referred to but not named. The Gegenfüsslerzellen [antipodals] originate in the same way as do the Keimbläschen (by free cell formation), and he refers to the Gegenfüsslerzellen as Antipoden des Keimbläschen. After fertilization, in some species mentioned, they do not develop farther and are then often dissolved. His Fig. 28^b shows all the nuclei in the typical embryo-sac as known today. Two free nuclei, after forming what botanists now call the fusion nucleus, are described as dividing, together with the protoplasm lying inside the embryo-sac wall and immediately around them, to form endosperm cells.

Strasburger (23) speaks of the origin of the *Keimbläschen* and their *Gegenfüsslerinnen* [antipodals]. But later (24) he first divides the *Eiapparat* [egg apparatus] into the *Ei* [egg] and *Gehülfinnen* [synergids].

Quite a modern description of a fully developed embryo-sac is given as follows: "On the one hand there is the egg with its two *Gehülfinnen*, and on the other the *Gegenfüsslerinnen*. Indeed, in the last division, two nuclei - one from the anterior end, one from the posterior - move out to the inner space of the embryo-sac, and we have seen that the two nuclei soon approach each other and fuse one with the other."

Warming (33) compared the nucellus with the angiosperm pollen sac and considers the latter the homologue of the *microsporange* and the former the homologue of the *macrosporange*. He admits that there is a difference between these two sporanges, for "in the nucellus, one single daughter cell [macrospore mother cell] serves for reproduction; in the pollen sac, there is a great number [microspore mother cells]." In each case he gives the daughter cell the name *cellule mère primordiale*.

The ovule Warming believes to be a transformed "leaf lobe;" "the nucellus is a new product of the value of an emergence." The angiosperm *integument* is thought to be

"a particular formation belonging to the ovular leaf and foliar in nature."

Treub and Mellink (28), Fisher (7), and Ward (31) continued the work of Warming by making a thorough study, in many different plants, of the development of the megaspore mother cell, of its divisions to form the megaspores and of the development of one megaspore into the embryo-sac. Mellink traced different stages in nuclear division in the embryo-sac.

Vesque (29, 30), Ward (32) and Guignard (9, 10) contributed to the knowledge of the embryo-sac in various monocotyledons and dicotyledons. [For work of Vesque in Labiatae see end of Historical Summary.]

Vesque and Warming held that the nucellus can be compared to the anther in that the megaspore mother cell is the homologue of the sub-epidermal cell of the pollen sac, which divides to form the spore mother cells. Hence they argued that one of the megaspores is a spore mother cell, and that when the nucleus of the megaspore which forms the embryo-sac has, by two divisions, resulted in forming four nuclei, each of these latter is a spore nucleus. To explain the eight nuclei present in the sac, they thought that two megaspores, as we should call them, have fused, and that the wall between has

disappeared, making two tetrad groups of spore nuclei within the sac. Then the egg would be a spore which never germinates; but, if it did so, any cells formed would be prothallial cells, including some archegonial cells.

On the other hand, Ward, in discussing to some extent the morphological nature of the megaspore which forms the embryo-sac, also that of the nuclei of the sac, assumes that the egg is homologous with the oosphere of Cryptogams. He says that in that case the other nuclei of the sac must correspond to the vegetative nuclei of a prothallus; the synergids are possibly two neck-cells of an archegonium, persisting because they aid in fertilization; the antipodals and the two polar nuclei must be cells of the prothallus. Whether the embryo-sac is composed of two tetrahedral groups forming two prothalli or is an eight-celled prothallus, depends, he says, on whether the vacuole-like space between the two groups be considered a wall or not.

This view of Ward's is somewhat similar to that held by Strasburger (25), who considered the embryo-sac to have developed from a megaspore whose nucleus by division has given rise to eight nuclei of a prothallium, one nucleus of which, the egg nucleus, represents an archegonium reduced to an oosphere.

The fusion of the polar nuclei, Strasburger thought,

results in the formation of a stronger, more active nucleus of the endosperm mother cell.

Strasburger (26) found that the nucleus of one male cell unites with the nucleus of the egg to form the nucleus of the first cell of the embryo. By this time the study of the chromosomes had advanced so far that their behavior during fertilization could be carefully noted. Hence, Strasburger's discovery was followed by his statement of the theory now quite firmly established: (1)The process of fertilization rests upon the union of the sperm nucleus carried into the egg with the egg nucleus, a principle most sharply formulated first by O. Hertwig (13); (2)The cytoplasm does not take part in the process of fertilization; (3)The sperm nucleus and the egg nucleus are genuine cell nuclei."

In his discussion of this theory, he says that "the daughter nuclei of the embryo nucleus, formed by the union of the sperm nucleus and egg nucleus, must have an origin half paternal and half maternal, a fact already alluded to in recent times by Nussbaum (21) and van Beneden (1)."

Nawaschin (20) discovered that the second male nucleus unites with one polar nucleus and these two with the second polar nucleus, to form the nucleus of the endosperm mother cell.

Guignard (11, 12), in an investigation entirely independent of that of Nawaschin, confirmed the results obtained by the latter. Numerous investigations since have proved that this fusion of the three nuclei, first spoken of as double fertilization and later as triple fusion, takes place in very many angiosperms, but not always in the same way. Sometimes the two polar nuclei fuse first; sometimes the antipodal and second male nucleus first, and sometimes this male nucleus fuses first with the polar nucleus which is a sister of the egg nucleus.

Strasburger (27) prefers to call the fusion of the first male nucleus with that of the egg nucleus generative fertilization, and the second nuclear fusion vegetative fertilization. He considers that generative fertilization is mainly to bring about the union of parental qualities, while the purpose of vegetative fertilization is simply to give a stimulus to growth. The polar nuclei are gametophytic, and, even though united with a male nucleus, their nature and the nature of the endosperm do not change; the fusion is for growth stimulus only.

Miss Sargant (22) supports Strasburger's view and adds that in fertilization which results in reproduction there is (1) a union of the parental qualities

and (2) a subsequent life history which repeats that of the parents. In triple fusion, (1) is brought about but not (2); hence this can not be generative fertilization, but rather vegetative fertilization. This writer thinks that the polar nucleus from the antipodal group, because of its usual excess of chromosomes, is of service in bringing about an easy and early disintegration of endosperm tissue after its work for the sake of the embryo is completed.

In this same article, Miss Sargant, with the permission of Miss Ethel Thomas, quotes the following hypothesis made, but then as yet unpublished, by Miss Thomas: In cross fertilization the endosperm, whose mother cell nucleus contains elements from both parents, can supply more satisfactory food for the embryo, whose nucleus also contains elements from both parents. Miss Sargant says that in this case triple fusion would be depending upon parental qualities and might be considered generative fertilization.

Very little work has been reported upon the embryo-sac development in Labiatae.

Vesque (29) studied and sketched the formation of the megaspores and embryo-sac in Sálvia praténsis. He found that the megaspore mother cell divides to form four or five megaspores, the number not being constant. The wall

between the first and second megaspores, counting from the micropyle, disappears and the resulting two-nucleate cell forms the embryo-sac proper, while the remaining megaspores form a cylindrical appendage of the sac . The part of the sac derived from megaspore number two enlarges and the micropylar end of the sac becomes pear-shaped. The other half of the sac remains joined to the first half by a small isthmus, back of which the cytoplasm bulges out to push against the bounding wall of the nucellus. Megaspore number one becomes two-nucleate.

The third, fourth and fifth megaspores grow to the size of what was megaspore number two. Megaspores numbers three and four divide transversely and longitudinally until they form a mass of cells which Vesque called the endosperm. He thought this endosperm "a sterile prothallus adapted to the nutrition of the embryo, the latter being a sexual product of five spore mother cells, one alone furnishing a fertile spore, and all the others undergoing many changes to aid in the development of this spore."

The walls between megaspores numbers two and three, and three and four have a tendency to gelatinize, but sometimes they seem to disappear without a fusion of their cytoplasm. Apparently, it is sometimes one, sometimes the other,of these megaspores that furnishes the endosperm.

In his résumé of the Gamopetalae, Vesque says that the megaspore mother cell divides into three (rarely), four or five special mother cells [megaspores]. Generally only one of these cells, the first, produces several cells of which but one fertile one forms the embryo. The second megaspore keeps its nucleus undivided, enlarges and forms a sort of "incubator chamber." The nucleus is none other than the old nucleus proper of the embryo-sac. The other megaspores keep their form and rest in the cylindrical, chalazal prolongation of the embryo-sac. He calls these cells the anticlines. Sometimes, but rarely, they produce a structure comparable morphologically to an embryonal vesicle. The endosperm of the greater number of the Gamopetalae (perhaps of all) is a sterile prothallus developed in one or more of the megaspores.

According to Billings (2), the megaspore mother cell in various Labiatae develops from a cell of the ovule, very probably from a cell of the nucellus, although this latter point is not definitely stated. The megaspore mother cell divides to form an axial row of four megaspores. The innermost megaspore of the four develops into an eight-nucleate embryo-sac of the ordinary type.

In his Fig. 1, Pl. XI, the mature embryo-sac of Lamium amplexicaule is shown. Two nuclei of the egg

apparatus and two of the antipodal nuclei are shown, with the large primary endosperm nucleus lying near the latter. The gourd-shaped sac, with the larger end toward the micropyle and with a slightly enlarged antipodal end, is surrounded by cells which are much alike in form and which, from the description, are cells of the integument.

The embryo-sac of Sálvia lanceolàta is shown in his Fig. 14, Pl. XIII. Two cells of the egg apparatus and two of the antipodals lie in the usual positions in the sac, and the very large endosperm nucleus lies midway between, and quite close to, each group, because the embryo-sac is short as compared with the length of the cells of the egg apparatus. The cytoplasm is quite evenly and finely reticulated. No large vacuoles are shown. The antipodals are very small as compared with the cells of the egg apparatus and lie in a prolongation of the sac at the chalazal end.

In his Fig. 15, Pl. XIII, the embryo-sac of the same plant is shown to lie in the end of the anatropous ovule, farthest from the exterior opening of the long micropyle and is approached by a very long fibro-vascular bundle extending from the funiculus. The great length of the micropyle and of the fibro-vascular bundle is caused by the great thickness of the integument

opposite the chalazal end of the sac. The embryo-sac is short as compared with the very long integument, and its form is gourd-shaped, with the chalazal end larger. A prolongation of the embryo-sac contains the antipodals and extends toward the fibro-vascular bundle approaching the sac.

The integument varies in thickness in different Labiatae, but a fibro-vascular bundle running through the ovule from the funiculus to the antipodal region of the sac is present in all the species examined.

MATERIAL AND METHODS.

The material was collected, for the most part, at Forest Hill Nursery, Madison, during the fall of 1911, from the last of September until about the middle of October.

The material at various stages was fixed in each of the three Flemming's solutions. All older ovaries were first separated from the calyx.

The usual method of hardening, embedding and sectioning was employed; the sections were cut five microns in thickness, and stained with Flemming's triple stain.

Satisfactory preparations were obtained from material fixed in each of the three solutions used. It is difficult to say which gave the best results.

Although Sálvia spléndens is a garden plant of wide distribution, it is a native of Brazil. This may account, in part, for the large proportion of imperfect embryo-sacs formed in the later stage of development in plants grown under our climatic conditions.

OBSERVATIONS.

The ovary of Salvia splendens is deeply four-parted and one ovule is formed within each part.

The ovule in an early stage (Fig. 1, Pl. I) is composed of cells of different shapes and sizes bounded by a layer of very regular epidermal cells, excepting at the base of the ovule at the side next the main stem; here there is the beginning of two rows of closely packed, large, irregular, epidermal cells. The nucellar cells just beneath have divided into two rows of closely packed cells. This condition of the epidermal and nucellar cells indicates the beginning of the integument. So far as this section shows, it has begun to develop on but one side of the ovule.

The ovule is orthotropous in form at this early stage (Fig. 1a, Pl. I).

Before the microspores have divided, the megaspore mother cell (Fig. 2, Pl. I) can be seen as a large, sub-epidermal cell in the distal portion of the nucellus. At this time the single integument has begun to bulge out around the base of the nucellus; it consists of several layers of cells much alike and resembling those of the nucellus immediately surrounding the megaspore mother cell,

The ovule has now bent over considerably and is

substantially campylotropous in form (Fig. 2a, Pl. I).

The megaspore mother cell (Fig. 3, Pl. I) grows rapidly, especially in length, and seems to be pushing backward into the interior of the ovule (Fig. 3a, Pl.I); at the same time there is an outward growth of the integument and an accompanying outward pushing of the layer of nucellar cells just about the megaspore mother cell because of increase in the dimensions of these nucellar cells. The integument, nucellus and megaspore mother cell keep pace with the growth of the cavity of the ovary.

While the integument is growing outward and closing around the nucellus, it leaves a narrow, cylindrical opening, the micropyle (Figs.3 and 3a, Pl. I).

In the ovule from which Fig. 3 was drawn, the enlarged megaspore mother cell has its nucleus near the center, but a trifle toward the micropylar end of the cell. The nucleus is in the spirem stage and rather faintly staining strands are abundant in the cytoplasm.

The megaspore mother cell later divides into two cells, which will be referred to as the micropylar daughter cell and the chalazal daughter cell. In some cases these daughter cells are equal or nearly so (Fig. 4, Pl. II); in other cases they are found much different in size (Fig. 5, Pl. II), in such instances the larger being at

the micropylar end. Since all of these sections show the condition after mitosis has occurred, it is difficult to say whether the daughter cells were or were not originally equal in size; but, in all probability, they were equal and the difference in size shown in Fig. 5 is due to a subsequent more rapid growth of the micropylar daughter cell.

In Figs. 6 and 7, Pl. II, the micropylar daughter cell has divided into what will be spoken of as the first and second megaspores, the first being nearer the micropyle. The chalazal daughter cell appears to have formed the third and fourth megaspores. Since all the megaspores are separated from each other by parallel walls, they form an axial row.

The nuclei and cytoplasm of the first and second megaspores stain very densely and are much shrunken. Their whole appearance suggests degeneration. The early degeneration of the first and second megaspores in each case, together with the instances of greater size of the micropylar daughter cell, indicates that the division of the micropylar daughter cell precedes that of the chalazal daughter cell.

In Fig. 6 the nuclei of the third and fourth megaspores do not appear. Each of these cells is larger than

either the first or the second megaspore. The cytoplasm of the third and fourth megaspores is vacuolated. In the fourth megaspore shown in Fig. 7 the nucleus and cytoplasm stain densely, but not so heavily as do those of the first and second megaspores. But the third megaspore, very much larger than any of the other megaspores, is very much vacuolated.

So far as these two figures are concerned, they prove that neither the first nor the second megaspore develops into the embryo-sac, but that the third megaspore probably does; my preparations of Sálvia spléndens do not enable me to determine the latter point with certainty.

At the stage of Fig. 7, what appears to be the third megaspore has grown to a much larger size than that ever reached by any of the other megaspores, apparently at the expense of the degenerating megaspores.

At these stages (Figs. 6 and 7), the cells in the distal portion of the nucellus are still in one layer and are very large, while those nucellar cells bordering the axial row of megaspores and lying near the chalazal end of the row are smaller, but, as shown in Fig. 6, are sometimes in two layers. The chalazal cells of the nucellus adjacent to the sac are becoming large and stain heavily. They lie in a rather curved line which is turned

back toward the funiculus.

The micropyle from now on is very slender; it is also long, for the integument is very thick in this region.

Beginning with the stages shown in Figs. 6a and 7a, Pl. II, the ovule is anatropous in form and continues so throughout the remainder of its history.

At the two-nucleate stage (Fig. 8, Pl. III), the embryo-sac has greatly elongated, the cytoplasm is finely vacuolated at either end while the region between has several large vacuoles, and the two nuclei have migrated, one to either end of the sac. All traces of the functionless megaspores have disappeared.

When the four-nucleate stage is reached (Fig. 9 Pl. III), the sac has increased still more in size; the two nuclei in either end of the sac are surrounded by most of the stainable cytoplasm of the sac, the region between the two nuclear groups being one large vacuole bounded, just within the membrane of the sac, by a thin, rather irregular layer of cytoplasm. The entire sac is spindle-shaped.

Excepting at the chalazal end of the sac, the nucellar cells, so far as they persist, are long, slender, much compressed and degenerated. Those in the distal portion of the nucellus have disappeared entirely. This disappearance of the nucellar cells in the micropylar region has

permitted the sac to protrude some distance into the now quite closed micropyle (Fig. 9a, Pl. III).

The chalazal cells immediately in contact with the sac are much enlarged, are densely stained, and are now (Fig. 9a, Pl. III) in close contact with several rows of cells whose cytoplasm stains more heavily than that in the adjacent cells. These rows can be traced back to the funiculus.

The eight-nucleate sac (Fig. 10, Pl. IV) shows further growth. A large vacuolated area now exists at the antipodal end of the sac and seems to have pushed the three antipodal nuclei toward the micropyle. These three, one here seen in tangential section, show no signs of degenerating. The two polar nuclei, quite close together, have taken a pink stain as compared with the red stain taken by the two antipodal nuclei shown in median section.

Each of these five nuclei has at least one nucleolus and lies in the rather dense but finely vacuolated cytoplasm of the sac. Several irregular, dark-staining masses are scattered throughout this cytoplasm.

The nuclei of the egg apparatus (one of which appears in the next section) take a very dense stain. They are large and lie in a mass of cytoplasm which is more vacuolated than that containing the antipodal nuclei and

polar nuclei but less than that at the chalazal end of the sac. None of these eight nuclei is surrounded by a cell wall or plasma membrane.

The nucellar cells surrounding the embryo-sac, excepting those at the chalazal end of the sac, have disappeared and in their stead closely pressed, integument cells lie next the sac. Some of these appear to be degenerating.

Fig. 11, Pl. V represents the mature embryo-sac. Two of the antipodal cells are shown in this section. They stain less heavily than do the synergids and egg and are finely vacuolated but not shrunken. Perhaps this, with their persistence, is an indication that the antipodals have no further growth of their own but that their function from now on is passive, perhaps that of conducting food from the chalaza to the other cells of the embryo-sac.

The very large, conspicuous body toward the center of the sac is probably the primary endosperm nucleus resulting from the fusion of the two polar nuclei.

The egg apparatus shows, in the section figured, two large nuclei, each in the inner end of its own long, slender mass of cytoplasm which extends from that nucleus to the micropylar end of the sac. Each cytoplasmic mass is surrounded by a thin membrane. The cell at the left is probably the egg and that at the right a synergid.

The cell and nucleus in the next adjoining section resemble the cell and nucleus just referred to at the right hand side and appear to have lain near the large vacuole shown in the figure at the left of the primary endosperm nucleus.

The sac is finely vacuolated throughout, and contains in addition long, irregular, clear spaces running here and there in the region of the egg apparatus from the neighborhood of the micropyle toward the primary endosperm nucleus. In none of my preparations has any trace of the pollen tube been found. The mature embryo-sac is rather gourd-shaped; the smaller end of the gourd is the antipodal end.

The very large chalazal cells of the nucellus, still larger than at earlier stages, connect the embryo-sac with the end of a fibro-vascular bundle which extends from these chalazal cells to and through the funiculus. The nucellar cells about the embryo-sac have entirely disappeared, leaving the embryo-sac surrounded only by the very thick integument except at the chalazal end, where the large nucellar cells remain.

DISCUSSION.

Vesque (29) reports finding that in Sálvia praténsis the megaspore nearest the micropyle, out of an axial row of four or five megaspores, develops typically into the embryo-sac, while the wall between the first and second megaspores breaks down and the cytoplasm of the second megaspore, remaining by itself, seems to form a chamber for food supply. In S. spléndens neither the first nor the second megaspore, counting from the micropyle, shows any tendency to develop into an embryo-sac.

In S. praténsis the functionless megaspores three and four are said to supply the endosperm; in S. spléndens the functionless megaspores disintegrate and disappear, probably absorbed by the embryo-sac, which appears to be formed from the third megaspore from the micropyle.

Only one division of the nucleus in the functioning megaspore in S. praténsis is mentioned; the two daughter nuclei come to lie, each in its own embryonal vesicle. The embryo-sac in S. spléndens finally has eight nuclei, as is common in angiosperms, and none of them lie in any vesicle; but, by the time the primary endosperm nucleus is formed, a membrane surrounds each of the nuclei of the egg apparatus and a membrane or else

a very thin wall surrounds each of the antipodal nuclei.

Vesque's report of the development of the embryo-sac and endosperm in S. praténsis differs so widely from those of others as to the development of the embryo-sac and endosperm in all other angiosperms that his observations for this report are not accepted as accurate. Besides, Strasburger (25) states very briefly that in S. praténsis he found the egg apparatus and antipodals having the usual origin and that the fusion of the polar nuclei occurs in the usual manner within the embryo-sac.

Billings (2), reporting upon a number of Labiatae, not including S. spléndens, finds that the megaspore at the chalazal end of the axial row of four megaspores develops into the embryo-sac. S. spléndens, according to my observations, seems to differ at this point, for, in the latter plant there are strong indications that the third megaspore, counting from the micropyle, develops into the embryo-sac. S. spléndens agrees with Billings' descriptions in the divisions of the nuclei of the embryo-sac and in the general form and relative positions of the antipodals, egg apparatus and endosperm nucleus.

The form of the embryo-sac is like that of Làmium amplexicáule (according to Billings), excepting that the

chalazal end of the sac in the plant last mentioned contains the primary endosperm nucleus, which makes the sac bulge at this region more than is the case in *S. spléndens*, where the primary endosperm nucleus is much nearer the micropylar end of the sac. The larger end of the sac in *S. lanceolàta* is the chalazal end, but in *S. spléndens* it is at the micropylar end.

The disappearance of the functionless megaspores and of the nucellar cells in the Labiatae described by Billings resembles that in *S. spléndens*. There is a strong resemblance, too, in the form of the ovule and in the great thickness of the integument at the micropylar end of the sac, hence in the resulting position of the sac, and in the length of the micropyle and of the fibro-vascular bundle. The enlarged nucellar chalazal cells in *S. spléndens* probably serve as a haustorium such as is described by Billings for several Labiatae.

The gourd form of the mature embryo-sac of *S. spléndens*, with the smaller end of the gourd toward the chalaza, is common to many of the Sympetalae [Coulter and Chamberlain] (5).

SUMMARY.

1. The megaspore mother cell in S. splendens is a hypodermal cell of the nucellus.

2. This mother cell enlarges and elongates much before dividing. In some cases, the daughter cells are practically equal in size and sometimes unequal. Because these cells in the sections observed had completed mitosis, it is difficult to say whether or not they were equal in size when first formed. However, there is a possibility that the cells at the time of mitosis were practically equal and that the micropylar daughter cell grows more rapidly than the chalazal daughter cell.

3. Each daughter cell appears to divide once, forming an axial row of four megaspores.

4. The third megaspore (counting from the micropylar end of the row) seems to develop into the embryo-sac. The other three degenerate. It is possible, however, that but three megaspores are formed, and that the third forms the embryo-sac, while the other two degenerate.

5. Three nuclear divisions occur in the typical way, followed by cell division into seven cells.

6. The two polar nuclei fuse before fertilization.

7. The mature sac is gourd-shaped, with the smaller

end toward the chalaza. The chalazal nucellar cells, grown very large and sometimes in one row, sometimes in two, connect the sac with a fibro-vascular bundle running into the funiculus. The nucellar cells, except at the chalazal end, disappear, and the embryo-sac lies embedded in a single, very thick integument.

The ovule, first orthotropous, then campylotropous, and lastly anatropous, in form, remains during the latter half of its development anatropous.

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DESCRIPTION OF FIGURES.

The figures were drawn at the level of the microscope stage; an Abbé camera lucida and the Leitz ocular 3 and oil immersion objective 1/16 were used throughout, excepting for Figures 1a, 2a, etc., for which objective number 3 was substituted for the 1/16. Fig. 1a is Fig. 1, together with the neighboring structures, all on a smaller scale. The same statement is true for Fig. 2 and Fig. 2a, etc.

a=antipodal nucleus - a^1, a^2, a^3 point out separate nuclei

ac=antipodal cell or antipodal

aw=anticlinal wall between megaspores

ch=chalazal cells

ch dc=chalazal daughter cell

e=egg

ea=egg apparatus

es=embryo-sac

f=funiculus

fvb=fibro-vascular bundle

i=integument

m=micropyle

m dc=micropylar daughter cell

meg=megaspore

m mc=megaspore mother cell

n=nucleus

nu=nucellus

o=ovule

ov=ovary

pen=primary endosperm nucleus

pn=polar nucleus

s=synergid

Plate I.

Figs. 1 and 1a. Early stage of ovule.

Figs. 2 and 2a. Early stage of megaspore mother cell.

Figs. 3 and 3a. Elongated megaspore mother cell.

Plate II.

Figs. 4 and 4a. Two cells substantially equal in size, derived from the division of the megaspore mother cell.

Figs. 5 and 5a. Micropylar daughter cell much larger than chalazal daughter cell.

Figs. 6 and 6a. First and second megaspores, and probably the third and fourth; the first and second degenerating.

Figs. 7 and 7a. Four megaspores; first and second degenerating; fourth not very active; third appears to be forming the embryo-sac.

Plate III.

Figs. 8 and 8a. Two-nucleate embryo-sac.

Figs. 9 and 9a. Four-nucleate embryo-sac; at upper left hand corner the drawing of part of an adjacent section shows the nucleus missing from 9.

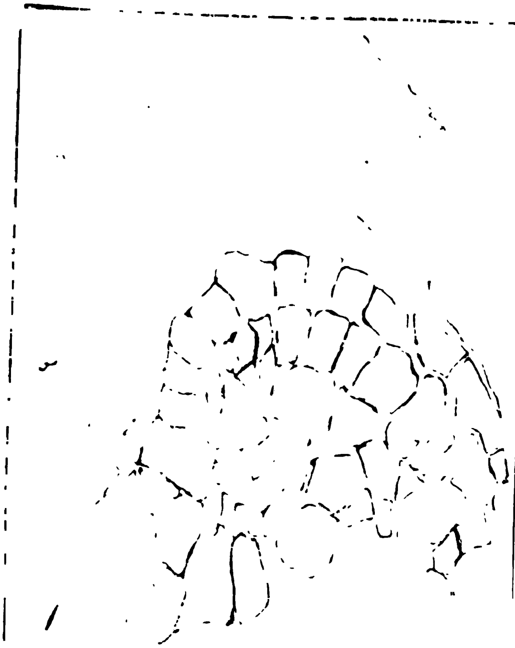
Plate IV.

Figs. 10 and 10a. Eight-nucleate embryo-sac.

Plate V.

Figs. 11 and 11a. Mature embryo-sac.

I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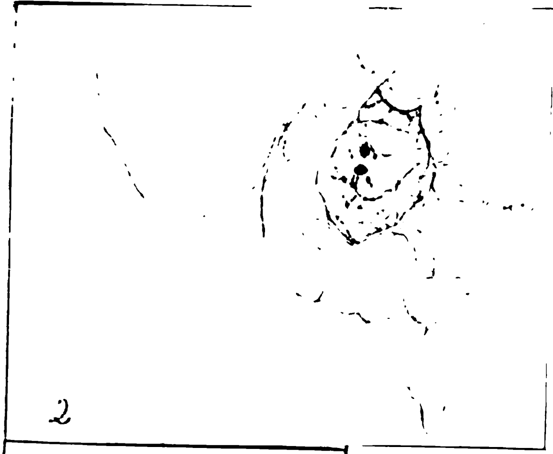
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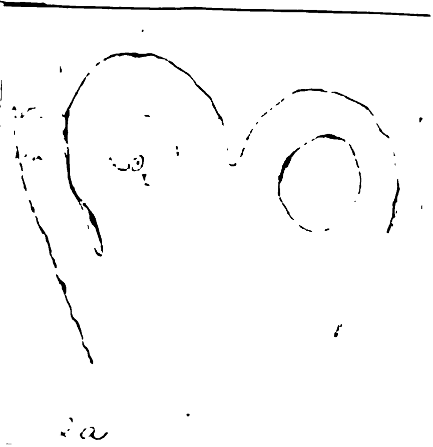
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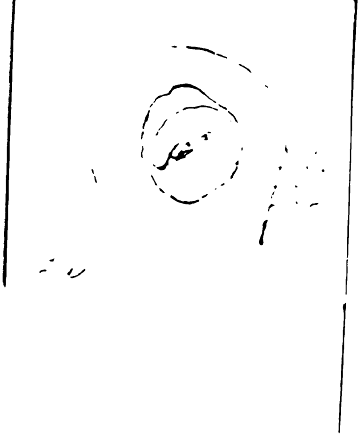

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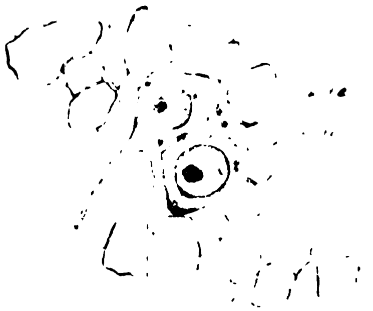

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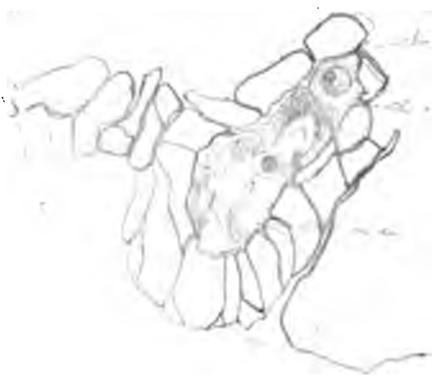

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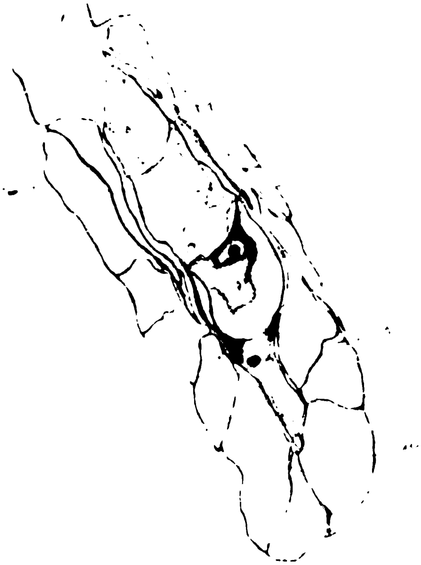

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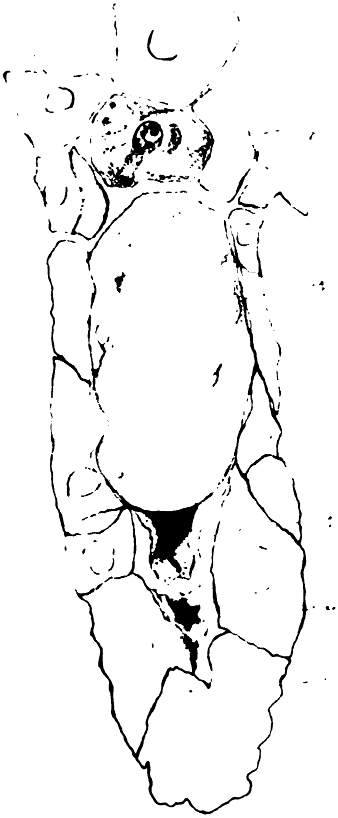

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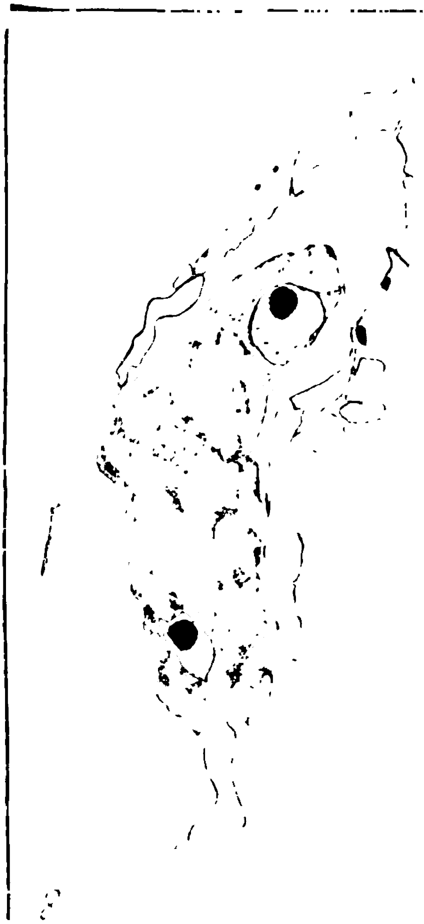

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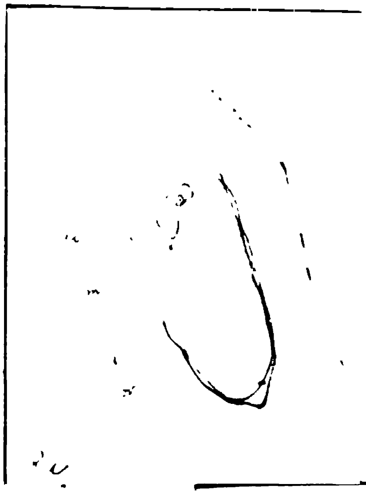

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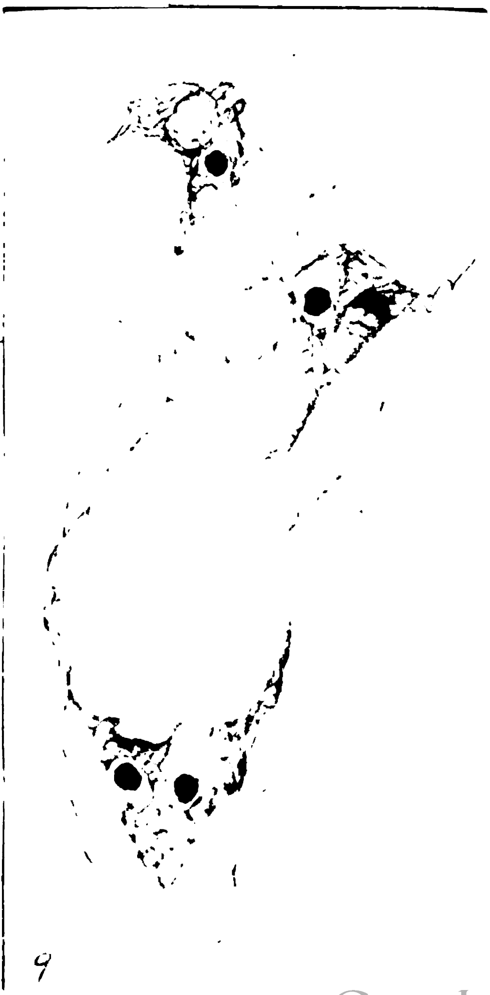

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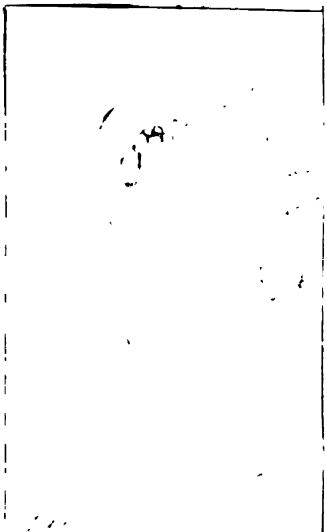

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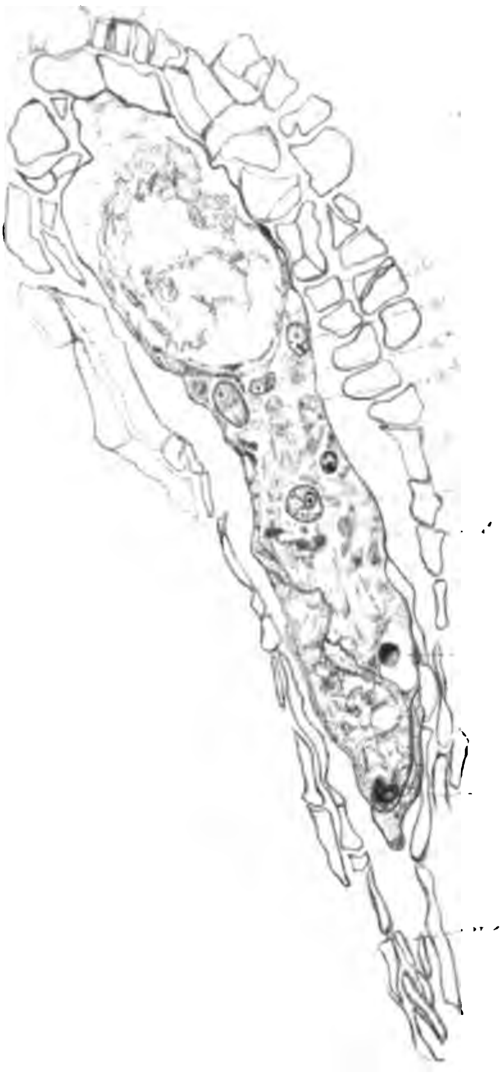

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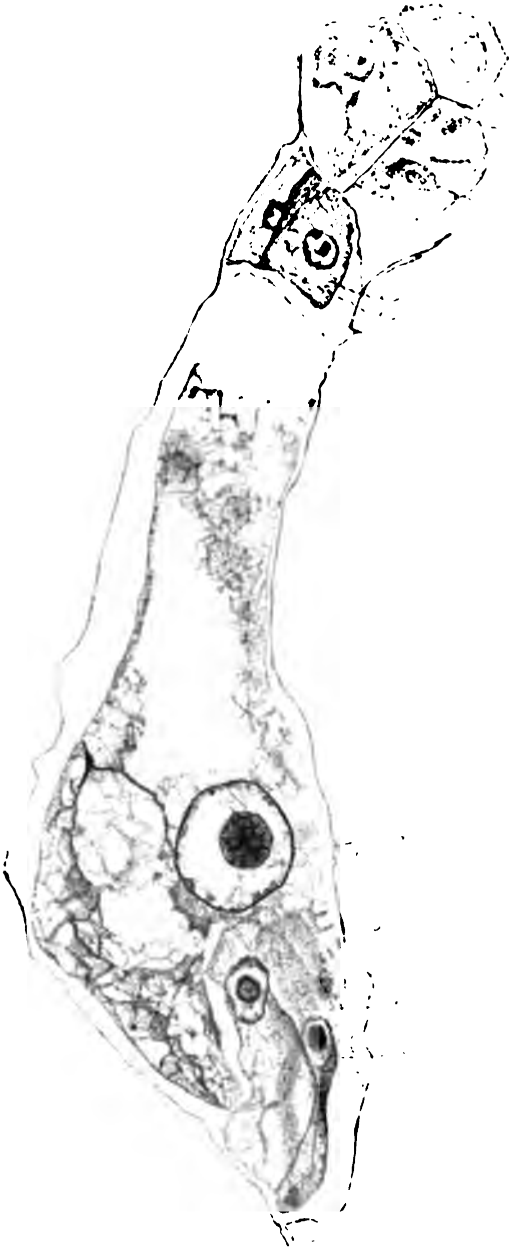

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Approved by C E Allen

Dated Lawrence Martin

April 26, 1913 J. B. Overton.

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